PATENT
Attorney Docket No.: 03715.0144-00000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	
Bernard Vacher et al.	)	Group Art Unit: 1625
	)	
Application No.: 10/518,394	)	Examiner: David K. O'Dell
	)	
Filed: December 17, 2004	)	
	)	
For: NOVEL ARYL-[4-HALO-4 (HETEROARYLMETHYLAMINO)-METHYL]-PIPERIDIN-1-YL]-METHANONE DERIVATIVES, METHODS FOR PRODUCTION AND USE THEREOF AS MEDICAMENTS	)	Confirmation No.: 8214

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

DECLARATION UNDER 37 C.F.R. § 1.132

I, Laurent Bardin, do hereby make the following declaration:

1. That I am a citizen of France, residing at 10, le Belvédère, Véries, CASTRES, France.

2. I have a Ph.D. in the Neuropharmacology from the University of Clermont Ferrand, (1996).

3. I have been working for over 10 years in the research laboratories of Pierre Fabre Medicament investigating and developing compounds for use in the treatment of drug dependence and pain.

4. I am person skilled in the art of developing and testing compounds for use in the treatment of drug dependence and pain, and that, as such, I am familiar with and understand the scientific literature in this area, as well as how a person skilled in this art would interpret results of experiments in this field of research, such as those described in Exhibit A of this Declaration.

5. I am familiar with the disclosure of the subject application, U.S. Patent Application No. 10/518,394, filed December 17, 2004, including the claims as amended in the accompanying Response and Amendment, including claim 10, directed to a method of treating pain by administering a compound of the invention.

6. The experiments described in Exhibit A of this Declaration were carried out under my supervision in the research laboratories of Pierre Fabre Medicament. These experiments demonstrate the *in vivo* analgesic effects of compounds of the invention in a formalin model of tonic nociceptive pain in rats.

7. The compound referenced as F 15599 in Exhibit A is (3-chloro-4-fluorophenyl)-(4-fluoro-4{[(5-methylpyrimidin-2-ylmethyl)-amino]-methyl}-piperidin-1-yl)-methanone, which is identical to compound 5 (1-5) of the subject application. The structure and synthesis of F 15599 are described in Example 5, on page 19 of the specification.

8. The figure in Exhibit A illustrates that F 15599 administered to rats either orally (A) or intraperitoneally (B) produced a dose-dependent inhibition in paw elevation and paw licking, two spontaneous pain response behaviors, following formalin injection into the hindpaw. This inhibition occurred in both the early and late response phases.

These results demonstrate that F 15599 has an analgesic effect in the *in vivo* rat formalin model of tonic nociceptive pain.

9. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: 25/11/2008

By: _____
Laurent Bardin, Ph.D.

Attached: Exhibit A (10 pages)

EXHIBIT A OF 37 C.F.R § 1.132 DECLARATION OF LAURENT BARDIN

INTRODUCTION

The prototypical 5-HT_{1A} agonist, 8-OH-DPAT produces analgesia in rats (Bardin et al., 2001), in the formalin model of tonic nociceptive pain: this effect is mediated by 5-HT_{1A} receptors and is not confounded by the 5-HT syndrome.

The localized injection of formalin into the hindpaw produces a characteristic biphasic electrophysiological (Puig and Sorkin, 1995) and behavioural response (Dubuisson and Dennis, 1977; Tjolsen et al., 1992). The first phase reflects a direct activation of sensory afferents, while the second phase reflects ongoing peripheral activity, inflammation and central sensitization (Coderre et al., 1993). Thus, this formalin model has been shown to be a suitable model of clinical inflammatory pain. The present study examined the potential analgesic activity after i.p. and p.o. administration of F15599, a selective, high efficacy 5-HT_{1A} receptor agonist.

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MATERIALS AND METHODS

Animals

Male Sprague Dawley rats (Ico: OFA SD [IOPS], Iffa Credo, l'Arbresle, France), weighing 160 to 180 g on arrival, were group-housed (five animals per cage) in an environmentally controlled room (temperature 21 ± 1°C; relative humidity 55 ± 5%) under a 12-h light dark cycle (lights on at 07:00 AM) with food and water freely available. A 5 days acclimatization period was allowed before animals were used in the experiments. The animals were transferred to the experimentation room on the day before the experiment began and were maintained under the same conditions as during quarantine.

All animals were handled in accordance with the guidelines of the Ethics Committee of the International Association for the study of Pain (Zimmermann, 1983) and procedures were approved by the institutional Ethics Committee. Animals were used only once and were sacrified immediately after the experiment.

Formalin test

All experiments were performed in a blind fashion in a quiet room, between 09:00 and 16:00, by a single experimenter. The formalin test was carried out as described (Bardin et al., 2001) in a clear plastic chamber, with a mirror placed underneath the floor at a 45°C angle to allow an unobstructed view of the paws. Each animal was first placed in the chamber for a 30 min habituation period. Thereafter, each rat received a 50 µl subcutaneous (s.c.) injection of diluted (2.5 % v/v/) formaldehyde into the plantar surface of the right hindpaw. Following this, the rat was returned to the chamber. The recording of

behavior started immediately and lasted for 5 min (early phase). The recording of the "late phase" started 23 min after the formalin injection, and also lasted for 5 min.

During each of these two 5 min periods, rats were observed every 30 sec, for the presence or absence of spontaneous pain behaviors, i.e.:

- The injected paw is elevated and not in contact with any surface
- The injected paw is licked

This observation cycle was repeated 10 times during each 5 min period; thus, the incidence of a particular behavior could vary from 0 to 10 for each of the two phases.

Drug administration

F15599 was synthetized at the CRPF and was dissolved in distilled water. F15599 or vehicle (0.9 % NaCl solution) was administered 15 min (i.p.) or 60 min (p.o.) in a volume of 10 ml/kg before the injection of formalin. Doses refer to the weight of the free base. The number of animals was 7 in each group.

The present series of experiments was carried out between February 2002 and July 2003.

Statistical Analysis

The results are expressed as the mean ± SEM and were analyzed by a one-way analysis of variance (ANOVA) followed, by Dunnett's post-hoc test. ED_{50} values were calculated for the mean score and from the percentage of animals showing significant inhibition of paw elevation and paw licking (i.e., scores lower than 9 for paw elevation and 5 for paw licking in early or late phase, respectively: these scores occurred in less than 5% of all vehicle/formalin-treated controls).

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The ED_{50} values and their associated confidence limits were calculated for the raw data by adjusting a 4 parameter logistic model to dose response curves, using the Mixed procedure of SAS 8.2 software for PC (Kallen and Larsson 1999). In addition, based on the percentage measure, the ED_{50} values and their associated confidence limits were calculated with the Litchfield and Wilcoxon probit analysis procedure, using the program described by Tallarida and Murray (1987). When less than two intermediate effects were observed, 0% and or 100% effects were transformed by means of Berkson's adjustement (Hubert, 1984) to permit the use of the Litchfield and Wilcoxon procedure.

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RESULTS

Administration p.o. (Fig. 1A)

P.o. administration of F15599 (0.01-2.5 mg/kg) induced dose-dependent inhibitory effects on both the paw elevation and paw licking, and this during both the early and late phases (paw elevation, early phase : $F [5.36] = 37.6$; $P<0.001$; paw elevation, late phase: $F [5.36] = 14.3$; $P<0.001$; paw licking, early phase: $F [5.36] = 36.1$; $P<0.001$; paw licking, late phase: $F [5,36] = 6.8$; $P<0.001$). The score of paw elevation in the two phases was significantly reduced from the dose of 0.63 mg/kg, with a total inhibition in the late phase only. The score of paw licking was also significantly reduced by F15599 in the two phases, from 0.16 mg/kg; total inhibition of paw licking was obtained in both phases at 0.63 and 2.5 mg/kg. ED_{50} values (calculated from raw scores) for inhibition of paw elevation were 0.43 and 0.22 mg/kg in the early and late phases, respectively, whereas ED_{50} values for paw licking were 0.14 and 0.06 mg/kg, respectively (Table 1). Based on the percentage of animals showing significant inhibition, ED_{50} values were 0.54, 0.44, 0.09 and 0.07 for inhibition of paw elevation in the early and late phases and for inhibition of paw licking in the early and late phases, respectively (Table 1).

Administration i.p. (Fig. 1B)

Similarly, i.p. injection of F15599 (0.01-2.5 mg/kg) produced significant effects (paw elevation, early phase : $F [5,36] = 17.9$; $P<0.001$; paw elevation, late phase : $F [5,36] = 43.4$; $P<0.001$; paw licking, early phase : $F [5,36] = 46.9$; $P<0.001$; paw licking, late phase : $F [5,36] = 47.9$; $P<0.001$). The score of paw elevation was near totally reduced in the two phases at the dose of 2.5 mg/kg, whereas total inhibition of paw licking was obtained in

both phases at 0.63 mg/kg. ED_{50} values (calculated from raw scores) for inhibition of paw elevation were 0.54 and 0.44 mg/kg in the early and late phases, respectively, whereas ED_{50} values for inhibition of paw licking were 0.09 and 0.07 mg/kg, in the early and late phases, respectively (Table 1). Based on the percentage of animals showing significant inhibition, ED_{50} values were 0.24, 0.26, 0.05 and 0.04 for inhibition of paw elevation in the early and late phases and for inhibition of paw licking in the early and late phases, respectively (Table 1).

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DISCUSSION

In the present study, i.p. or p.o. administration of F15599 (0.01-2.5 mg/kg) potently and dose-dependently inhibited formalin-induced paw elevation and paw licking during both phases. The dose of 2.5 mg/kg produced a complete suppression of all four parameters and particularly, paw elevation in the early phase, a parameter particularly resistant to various classes of analgesic drugs (Bardin et al., 2003).

In addition, though the first and the second phases induced by formalin injection involve different mechanisms and respond differently to some analgesic treatments (Coderre et al., 1993; Jourdan et al., 1999), the ability of F15599 (after i.p. or p.o. administration) to inhibit in an identical pattern these two phases is consistent with evidence that activation of 5-HT_{1A} receptors induces central analgesics effects (Bardin et al., 2001). The comparison of the ED_{50} values obtained by the two routes of administration indicates that the p.o. over i.p. ratio is close to unity, suggesting that the compound has a very good oral bioavailability in rats.

In summary, F15599 exerts a potent and efficacious analgesic action in the formalin model of tonic nociceptive pain in rats, .

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Table 1: Ability of F15599 administered p.o. and i.p. to reduce paw elevation and paw licking in the formalin pain test in rats. ED_{50} values in mg/kg.

	Paw Elevation ED_{50} (95% CI)		Paw licking ED_{50} (95% CI)	
	Early Phase	Late Phase	Early Phase	Late Phase
Behavioral Score				
F15599 p.o.	0.43 (0.18-1.04)	0.22 (0.09-0.56)	0.14 (0.10-0.18)	0.06 (0.02-0.14)
F15599 i.p.	0.54 (0.43-0.70)	0.44 (0.31-0.61)	0.09 (0.05-0.15)	0.07 (0.05-0.11)
% animals affected				
F15599 p.o.	0.12 (0.03-0.45)	0.02 (0.003-0.15)	0.06 (0.02-0.24)	0.05 (0.01-0.16)
F15599 i.p.	0.24 (0.09-0.61)	0.26 (0.18-0.55)	0.05 (0.01-0.16)	0.04 (0.01-0.11)

CI: confidential interval

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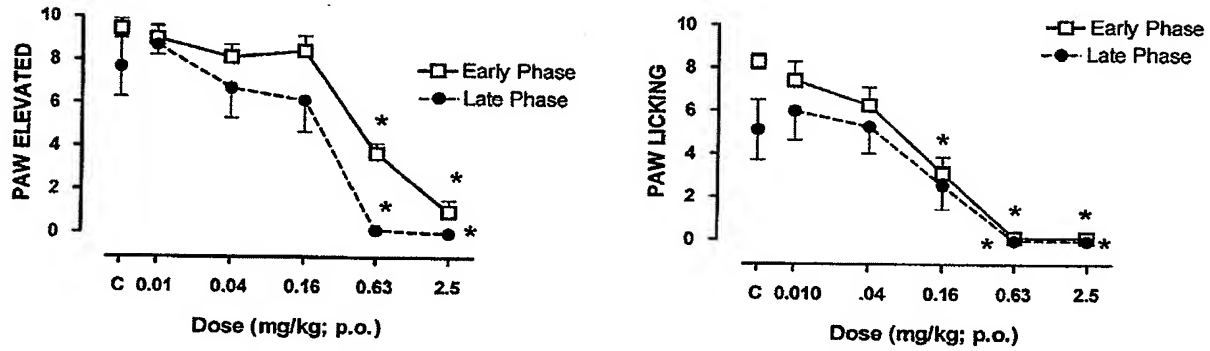
A



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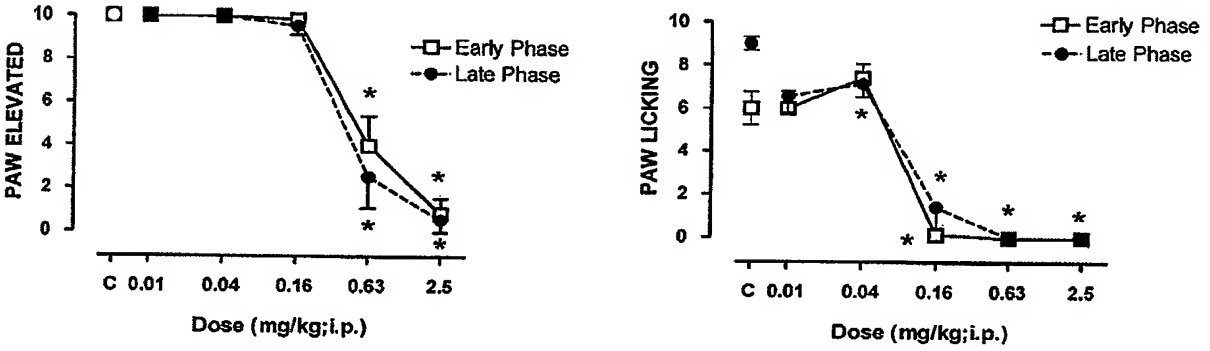

Figure: Dose-dependent effects of F15599 (0.01-2.5 mg/kg) administered p.o. (A) or i.p. (B) on the paw elevation and paw licking during the early (i.e., 0-5 min: open square) and late (i.e., 22.5-27.5 min: solid circle) phases of the formalin test in rats. F15599 or its vehicle was administered i.p. 15 min or p.o. 60 min before the formalin injection. Values represent mean ± S.E.M. score (maximal score = 10) of seven animals. * $P < 0.05$ (Dunnett's post-hoc test following significant one-way ANOVA) compared with vehicle-controls (C).